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Review Article

The more the messier: centrosome amplification as a novel biomarker for personalized treatment of colorectal cancers

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Abstract

Colon cancer is currently the third most common cancer and second most fatal cancer in the United States, resulting in approximately 600,000 deaths annually. Though colorectal cancer death rates are decreasing by about 3% every year, disease outcomes could be substantially improved with more research into the drivers of colon carcinogenesis, the determinants of aggressiveness in colorectal cancer and the identification of biomarkers that could enable choice of more optimal treatments. Colon carcinogenesis is notably a slow process that can take decades. Known factors that contribute to the development of colon cancer are mutational, epigenetic and environmental, and risk factors include age, history of polyps and family history of colon cancer. Colorectal cancers exhibit heterogeneity in their features and are often characterized by the presence of chromosomal instability, microscopic satellite instability, or CpG island methylator phenotype. In this review, we propose that centrosome amplification may be a widespread occurrence in colorectal cancers and could potently influence tumor biology. Moreover, the quantitation of this cancer-specific anomaly could offer valuable prognostic information and pave the way for further customization of treatment based on the organellar profile of patients. Patient stratification models that take into account centrosomal status could thus potentially reduce adverse side effects and result in improved outcomes for colorectal cancer patients.

Keywords: centrosome amplification, colorectal cancer, chromosomal instability, aneuploidy, biomarker, prognostic

Introduction

The colon, or large intestine, carries digested food from the small intestine to the anus. Colon cancer involves any epithelial neoplasms, or abnormal growth in the colon tissue. It is a common epithelial neoplasm that affects about 1.4 million newly diagnosed persons globally per year with over 600,000 annual fatalities[1,2]. Additionally, millions of men and women identified with colon polyps alone are at high risk for colon cancer. Other major risk factors include family history of colon cancer and age. Twenty percent of those diagnosed with colon cancer have familial or congenital mutations in genes that accelerate carcinogenesis to an early age onset[2]. The remaining 80% tend to develop colon cancer later in life and do not exhibit any obvious genetic causes; this suggests environmental, epigenetic or other mutational factors playing a causal role in the

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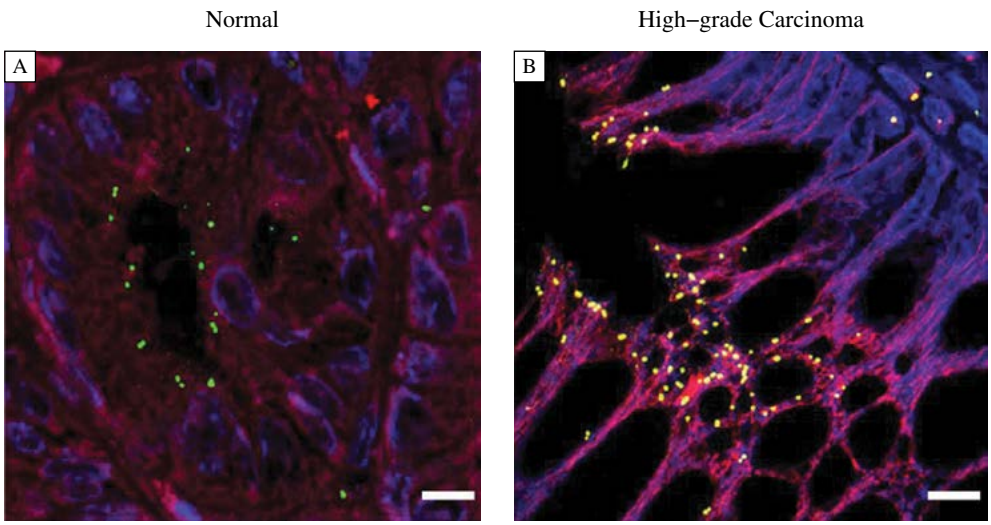

***Fig. 1* Colorectal carcinomas show rampant centrosome amplification.** Representative immunofluorescence confocal micrographs showing centrosome profiles in normal adjacent (left panel) and high-grade carcinoma (right panel). Centrosomes and microtubules were visualized by immunostaining for γ-tubulin (green) and α-tubulin (red), respectively. DNA was 4,6-diamidino-2-phenylindole (DAPI) stained (blue). Both numerical and structural centrosome amplification are evident in the high-grade carcinoma. Scale bar, 5 μm. A: normal; B: high-grade carcinoma.

etiology of these colon cancers[2]. Colon carcinogenesis is associated with frequent mutations in several pathways that include phosphatidylinositol 3-kinase (PI3K), adenomatous polyposis coli (APC), p53, F-box and WD repeat domain containing 7 (Fbxw7), transforming growth factor-β,and *K-RAS*. Screening for colon cancer is recommended at age 50, or 10 years younger than the age at which a family member was diagnosed with colon cancer. Colon carcinogenesis is a slow and stepwise process that can take decades. It is generally believed that normal colon tissues acquire driver mutations and then develop into hyperplasia. Hyperplastic tissues may subsequently progress into early adenomas, then into intermediate adenomas and finally into carcinomas that may exhibit additional gene mutations, oncogene activation, loss and gain of chromosomes, and/or centrosome amplification (CA)[2]. Colon carcinomas are adept in spreading to the liver and lungs[2]. Colon cancer metastases may be either synchronous (two or more carcinomas coexisting at initial diagnosis) or metachronous (consequent development of a second carcinoma after initial diagnosis of primary carcinoma). Approximately 20% of patients are found to have synchronous metastasis at the time of initial colon cancer diagnosis and more than 30% of patients develop metachronous metastasis[3] .

Colon cancer diagnosis, staging and current therapies

Most people are asymptomatic early in the disease. However, symptoms of a change in bowel habits, blood in stool, dark tarry stool and stomach pains can be caused by colon cancer. Colon cancer is exclusively diagnosed by performing a colonoscopy with biopsies. Guidelines related to colonoscopy have been determined by several organizations such as American Cancer Society, American College of Gastroenterology and the American College of Radiology[4]. Gastroenterologists only perform a colonoscopy when clinically indicated. The only blood test available for colon cancer patients is the carcinoembryonic antigen test, which is not recommended for screening because of low sensitivity and specificity, particularly in the early stages of neoplastic disease. Colonoscopy involves insertion of a long, flexible tube with a tiny video camera into the rectum and its guided movement throughout the colon. Pictures are taken and any polyps, or growths, found in the colon are removed for biopsy. Some polyps are not harmful (benign) while other polyps are cancerous (adenomatous) or can turn into cancer (hyperplastic). A follow up colonoscopy is dependent upon the initial colonoscopy results and family history: a normal colonoscopy result requires a routine follow-up in 10 years, unless the patient has a family history of colon cancer or colon polyps. If the patient had family history of colon cancer but their colonoscopy was normal, or if they were found to have one or two tubular adenomas less than 10 mm, they would require a repeat colonoscopy in 5 years. In the event a tubular adenoma larger than 10 mm in size, three to ten tubular adenomas or an advanced polyp was discovered on colonoscopy, a repeat colonoscopy is required in 3 years. A much shorter follow up period (less than 3 years) is recommended if a large

polyp which could not be completely removed or more than 10 polyps were found on first colonoscopy, or if the patient had any previous surgery to remove colon cancer.

For colorectal cancer (CRC), the stage is based on how far the cancer has grown into the wall of the intestine, if it has reached nearby structures, and if it has spread to the lymph nodes or distant organs. The stage of a cancer is one of the most important factors in determining prognosis and treatment options. Stage I and II colon cancers are still localized and therefore have high cure rates, rating 80%-95% for stage I and 55%-80% for stage II[2]. Cure rates drop significantly in stages III and IV of colon cancer, when the cancer has advanced to lymph nodes and metastasized to other organs, to 5%-10%[2]. Colon cancer patients in the advanced stages of colon cancer have a life expectancy of 5 years at most and unfortunately only around 40% of CRCs are found at the early, more curable stages[2]. When colon cancer is diagnosed, there are only two treatment options: surgery and/or chemotherapy with radiation. Surgery involves partial removal or total removal of the colon[1]. The regimen for colon cancer chemotherapeutic medication is commonly 5-fluorouracil (5-FU) (an irreversible inhibitor of thymidylate synthase) in combination with leucovorin (a reduced folate that increases thymidylate synthase inhibition), irinotecan (an inhibitor of Topoisomerase I), and oxaliplatin (a third-generation platinum derivative)[1]. Newer medications such as cetuximab and panitumumab specifically target and inhibit epidermal growth factor receptor (EGFR), a signaling cascade important for the growth and division of cancer cells[1]. Bevacizumab, ramucirumab and ziv-aflibercept are drugs used for colon cancer that target vascular endothelial growth factor and inhibit angiogenesis. Unfortunately none of these treatment types are universal for all colon cancer patients; the treatment responses vary dramatically and both treatment options can cause physiological and psychological side effects on the patient[1]. There are currently no blood tests that can diagnose colon cancer, nor are there drugs that directly target colon cancer cells alone. This has been problematic for both the patient and gastroenterologists as colonoscopy is an invasive test and many patients tend to defer a screening colonoscopy or routine colonoscopy owing to risks involved, psychological reasons and high cost.

In order to provide earlier diagnosis, improved treatment and overall improved prognoses, the molecular aspects of colon cancer must be addressed. Molecular approaches include developing methods to identify high-risk groups (e.g., molecular markers to indicate high colon cancer risk), identifying an effective use of prevention (such as natural, dietary and drug products), developing a non-invasive marker that can be readily and reliably detected *via* a clinically-facile assay as an alternative screening method, and addressing unmet clinical needs for therapy in order to establish more tailored treatments[2].

Heterogeneity in colorectal cancers

A large body of evidence suggests that CRCs display significant differences in clinical presentation and molecular characteristics depending on the driver and nondriver mutations present, somatic polymorphisms in the patient, cell type that the tumor originated in, external influences such as lifestyle, the clonal composition of the tumor, immune status and inflammatory context that the tumor occurs in. Moreover, the relationship between individual genetic aberrations and clinical behaviour is seldom direct or clear cut. Heterogeneity in tumor biology explains the oft-observed variations in responses between individuals given targeted treatments. For example, it is estimated that only 35% of patients with wild-type *KRAS* actually respond to anti-EGFR therapy. A mind-boggling array of mechanisms underlying anti-EGFR therapy resistance has been recently uncovered including mutations in *KRAS* and *BRAF* and upregulation of other receptors-all of which determine the extent of patient response. Since routine testing for all known drug response modifiers is impractical, decision-making for anti-EGFR therapy is still based on assessing mutational status of *KRAS* alone. Recently, some groups have attempted to identify distinct subtypes of CRCs based upon gene expression signatures that have impressive prognostic and predictive value[5-10]. These studies have lent credence to the notion that different CRC subtypes should perhaps be viewed as distinct disease entities with different vulnerabilities with respect to therapeutic modalities. Gene expression-based assays, however, bear the serious drawbacks of being cost-prohibitive, time consuming and requiring specialized expertise to carry out and interpret. Therefore, in addition to histological characteristics and disease stage, novel prognostic and predictive biomarkers that can be readily and cost-effectively determined in the clinic are direly needed for better patient stratification and more optimal therapeutic decision-making.

Genetic and epigenetic changes in colon cancer

One of the hallmarks of cancer is the widespread prevalence of genomic instability[11]. Cytogenetic studies such as karyotyping and fluorescence in situ hybridization of colon cancers have shown a high degree

of genomic instability and aneuploidy. Mutations in pathways that include PI3K, APC, p53 and *K-RAS* are believed to often trigger colon carcinogenesis. These colon cancer genes also bear a causal relationship to genomic instability. Conversely, genomic instability itself displays a feedback-type relationship with colon cancer gene mutations in experimental settings, as demonstrated in transgenic mouse models with high genomic instability[2]. In colon cancer, tumors frequently exhibit three forms of genetic or epigenetic changes: chromosomal instability (CIN), microsatellite instability (MSI) and CpG island methylator phenotype (CIMP)[12-14]. CIN is the most common type of genomic instability found in colon cancer, and occurs in 80-85% of cases[15-16]. The less common MSI occurs in between 10%-15% of colon cancers[17] while CIMP occurrence can vary from 12%-25%[18]. These subtypes have different predictive and prognostic impact for patients, as they are often associated with specific mutations. They are also not mutually exclusive as colon cancers often display characteristics of more than one genetic and epigenetic subtype. Subtype definition can therefore be key to selection of optimal therapeutic regimen and for more accurate disease prognosis.

CIN occurs when there is a high rate of gain or loss of either whole chromosomes or parts of chromosomes; this often results in aneuploidy and loss of heterozygosity (LOH)[1,19]. CIN can arise from chromosomal segregation errors, CA or telomere dysfunction. In addition to karyotypic changes, certain mutations are commonly found in CIN-positive CRCs, e.g., mutations in the tumor suppressor *APC, p53, SMAD2, SMAD4* or *DCC*[20]. Despite efforts to harness their prognostic value, none of these mutations are currently in use for prognosis in the clinic.

MSI is characterized by a loss of DNA mismatch repair mechanisms either owing to mutations in mismatch repair genes or hypermethylation of mismatch repair gene promoters leading to their downregulation. DNA mismatch repair genes normally maintain genomic integrity by repairing accidental changes in DNA that occur during replication. Mismatch repair deficiency is typified by changes in the length of microsatellite regions, which are mono-, di- or tri-nucleotide repeats found in several genes; these changes cause somatic mutations, including frameshifts, resulting in the production of truncated or non-functional versions of several key proteins involved in CRC development (such as PTEN, BAX). MSI is associated with frequent DNA damage, which can result in gross chromosomal rearrangements including translocations, duplications, inversions, or deletions. Events that cause the MSI phenotype tend to take place mainly in G1, S, and G2 phases, whereas CIN becomes apparent during mitosis (due to defects in the spindle, the function of the spindle assembly checkpoint, or defective kinetochores) or in a pre-mitosis phase. Tumors with MSI tend to present with higher amounts of tumor-infiltrating lymphocytes and lower differentiation status[21]. Determination of MSI status can be challenging due to the need to identify the best markers for assessing the microsatellite length differences. A recently developed marker panel with five mononucleotide repeats is currently used widely for this purpose[22]. Although MSI status is currently not used for disease prognosis, patients with MSI-positive tumors show better survival than MSI-negative or CIN-positive tumors. This effect is further altered by the presence of other mutations in the genome.

Tumors with CIMP are characterized by epigenetic instability owing to aberrant methylation of DNA CpG islands in ~5% of genes[23-24] which significantly exceeds the number of mutated genes[25]. Normally, these islands are located in promoter regions or the first exon of 70% of human genes and are unmethylated unless they are linked to imprinted genes or genes located on the inactivated X-chromosomes. Studies have revealed that the aberrant methylation patterns characterizing CIMP are established early during colorectal tumorigenesis[26] and that generally, there is an inverse relationship between CIMP and CIN[27-28]. CIMP may lead to MSI in certain instances because the majority of sporadic MSI-positive tumors arise *via* epigenetic silencing of expression of *MLH1* gene, a key regulator of the mismatch repair pathway. Importantly, CIMP-positive CRCs are usually associated with better prognosis[29], although patients with CIMP-positive CRC do not benefit from 5-fluorouracil-based adjuvant chemotherapy regimens[30]. Currently, there is no standard set of promoter regions for the assessment of CIMP status and three widely-used marker panels measure methylation levels using different sets of chromosomal regions leading to incomparability of results[31-33]. Moreover, these panels have different sensitivities and specificities and there is no consensus on how the results should be interpreted.

Origin of CIN: Extra centrosomes are identified as culprits

Though the exact mechanisms underlying CIN are still being uncovered, CA has been observed as a major underlying mechanism for CIN, aneuploidy and multipolar mitoses[34]. CA refers to the presence of more than two centrosomes at the onset of mitosis (numerical amplification) or the presence of centrosomes that

exhibit abnormalities in structure such as an increased volume (structural amplification). CA was observed over a century ago by the keen eyes of Theodor Boveri who surmised that this organellar surfeit may be responsible for the malignant nature of cancer cells. Indeed, CA is so widespread that it is considered as a hallmark of cancer. CA correlates strongly with CIN, karyotypic heterogeneity, and other phenotypes associated with aggressive tumor behavior[35-36]. The presence of supernumerary centrosomes in cancer cells means that sister chromatids may now attach to more than one centrosome during mitosis (merotely). Extra centrosomes may result in formation of a multipolar spindle that can prove lethal because it can lead to subsequent anaphase catastrophe or multipolar mitosis[37]. Cancer cells, however, cleverly sidestep these calamities by rapidly engaging "centrosome clustering mechanisms" that efficiently corral supernumerary centrosomes, along with their occasional merotelic attachments, into two polar groups to eventually assemble a pseudobipolar mitotic spindle[38-39]. In addition to evading cell death, centrosome clustering offers the collateral benefit of maintaining a low level of whole chromosome missegregation (CIN) that allows cancer cells to continue shuffling their karyotypes at a "tolerable" level (that does not jeopardize their viability) and provides the genetic variation that would allow selection of superiorly aggressive phenotypes[40]. Thus, centrosome clustering, a process that repeats itself mitosis after mitosis, is a strategy for both managing extra centrosomal load as well as for maintaining an optimal and persistent level of CIN. Thus, CA is a potent driver of both tumor-igenesis[41] and aggressive cancer phenotypes[36,42-43]. Supernumerary centrosomes are known to result in the nucleation of excess microtubules and disrupt the polarity and cytoarchitecture of tissues. Extra centrosomes are thus major contributors to mechanisms underlying genomic instability as well as loss of tissue architecture noted in cancers[19] .

Studies have demonstrated that CA is a very early event that occurs widely in preinvasive lesions of the uterine cervix, prostate and breast[44]. In colon cancer, CA is detected as early as low-grade dysplastic lesions of colorectal adenoma to adenocarcinoma sequences. CA is more frequent in carcinoma than adenoma and is associated with higher histologic grade of both dysplastic as well as invasive lesions[19]. In a study that determined the number of centrosomes per cell in for-malin-fixed paraffin-embedded colon tissues (21 normal colonic epithelium samples, 27 low-grade intraepithelial neoplasia samples, 16 high-grade intraepithelial neoplasia samples and 33 invasive adenocarcinoma samples), the authors found that the low- and high-grade intraepithelial neoplasia samples and invasive adenocarcinoma samples bear significantly higher centrosome numbers per cell than normal colonic epithelium[45]. They also found that the regular, apical-oriented distribution of centrosomes in normal colon epithelial cells was highly disturbed in all the neoplastic samples with invasive adenocarcinoma samples displaying a complete loss of centrosome orientation and polarized location, which is an essential feature of the epithelium's tissue architecture. Furthermore, steric distortion of the gamma-tubulin signals increased in the neoplastic samples relative to the normal colon epithelium indicating aberrations in centrosomal structure. The striking observation that both numerical and structural changes in centrosomes were already evident at the transition of normal epithelium to low-grade intraepithelial neoplasia, suggests that CA may represent a very early event in the carcinogenesis of CRC and may even indicate a causative role of CA in malignant transformation and loss of tissue architecture in the colon.

Interestingly, a study that compared the cytogenetic profiles of mismatch-repair-deficient diploid versus mismatch-repair-proficient aneuploid CRC cell lines using comparative genomic hybridization and spectral karyotyping, revealed a greater extent of chromosomal imbalances in aneuploid cells lines compared to diploid tumor cell lines. Importantly, this study uncovered the exclusive occurrence of CA and CIN in aneuploid cell lines while diploid tumor lines possessed centrosomes that were normal in number and appearance[46]. These data thus support a role for CA in the generation of CIN and aneuploidy in CRCs.

Many causes, common consequence: the pathways that potentially underlie the etiology of CA in colorectal cancers

p53 pathway

Deregulation of the tumor suppressor p53 plays a major role in the pathology of a wide range of cancers as evidenced by the fact that the p53 protein is inactivated in more than half of all human cancers[47-49]. p53 is a transcription factor that regulates expression of a number of genes. In addition, p53 localizes to centrosomes[50-54] and in tumors, loss or mutational inactivation of p53 is associated with abnormal amplification of centrosomes[55]. Loss of p53 can lead to genomic instability through both deregulation of the centrosome duplication cycle and cytokinesis failure[56,57]. Since p53 is involved in p21-dependent cell cycle arrest and/or cell death after failed mitosis, loss of p53 also permits tolerance of significant levels of aneuploidy and

polyploidy[56,58-60]. In CRCs, numerical and structural centrosomal aberrations are observed as early and stable events[45] . Importantly, researchers have found both CA and CIN in several aneuploid CRC cell lines; by contrast, diploid CRC cell lines harbored normal numbers of centrosomes whose structures were normal[61], suggesting that CA may be causatively linked to CIN in CRC. In another study, targeted inactivation of p53 in colorectal HCT116 cells and in primary human fibroblasts led to a 3.5-fold increase in tetraploidization[62]. Tetraploid cells are notorious for engendering aneuploidy, especially if they undergo multipolar mitoses subsequently[63]. p53 is involved in centrosome clustering and preventing multipolar mitoses in tetraploid cells[64]. Thus, loss of p53 is implicated in both induction of CA and generation of genomic instability in CRCs.

The SASS6 pathway

Deregulated expression of a key protein involved in centriole duplication, spindle assembly abnormal protein 6 homolog (SASS6) was recently demonstrated in primary CRCs[65] where an increase in SASS6 mRNA and protein expression was observed. In DLD-1 colon cancer cells, SASS6 overexpression induced CA, mitotic abnormalities such as chromosomal misalignment and lagging chromosomes, and CIN. SASS6 overexpression was also associated with anaphase bridge formation, a type of mitotic structural abnormality, in primary CRCs. This study thus suggested that SASS6 overexpression may be involved in the occurrence of CA and CIN in CRCs.

The *β*-catenin pathway

Mutations in the locus encoding β-catenin are believed to be early events in the pathology of colon cancer[66,67]. β-catenin normally plays a crucial role in the Wnt signaling pathway wherein it binds the T-cell factor to form the β-catenin-T-cell factor complex to transcriptionally regulate a suite of downstream genes[68]. β-catenin also has an essential role in cell-cell adhesion. Normally, β-catenin levels are tightly controlled by a destruction complex comprising APC and axin, which facilitates the phosphorylation of β-catenin by casein kinase I and glycogen synthase kinase 3β (GSK3β); this leads to the ubiquitylation of β-catenin and its proteasome-mediated degradation[69-74]. Mutations in the casein kinase I and GSK3β phosphorylation sites of β-catenin are often found in human tumors and result in (i) stabilization and accumulation of β-catenin, and (ii) mis-expression of genes regulated by the β-catenin-T-cell factor complex[66,75]. Research has also identified β-catenin as a centrosomal component that interacts with centrosomal proteins to regulate centrosome separation[76]. Interestingly, CIN is common in colon cancers with stabilized β-catenin[77,78]. A study[79] the role of mutant β-catenin in the formation of extra centrosomal structures in (a) HCT116 cells which have one wild-type allele of β-catenin and one mutant allele of β-catenin that cannot be phosphorylated by casein kinase I and GSK3β[75], and (b) cell lines derived from these parental cells in which either the wild-type allele or the mutant allele was deleted by somatic cell gene targeting[80]. This study showed that expression of stabilized mutant forms of β-catenin induces formation of extra centrosomal structures in HCT116 cancer cells. Removal of the mutant β-catenin allele from HCT116 cells significantly decreased the number of abnormal ɣ-tubulin structures in asynchronous and S-phase-arrested cells, and decreased amplification of SAS-6-positive centrioles during S-phase arrest. Thus, accumulation of a mutant form of β-catenin found in early stages of many cancers[67] may directly induce centrosome aberrations.

The Met pathway

Met, also known as the hepatocyte growth factor receptor, is a membrane-localized receptor tyrosine kinase protein that is essential for embryonic development and wound healing. Hepatocyte growth factor is the only known ligand of the MET receptor[81]. MET is normally expressed by cells of epithelial origin, while expression of hepatocyte growth factor is restricted to cells of mesenchymal origin. Upon hepatocyte growth factor stimulation, MET signaling induces invasive growth. The oncogenic ability of aberrant Met signaling was thought to mainly rely on its mitogenic and anti-apoptotic effects. Recently, accrued evidence, however, suggests that genomic instability may be a crucial factor in MET-induced tumorigenesis. A study in HCT116 colon cancer cells showed that expression of a constitutively active version of Met induced (a) CA mainly *via* centrosomal overduplication during the cell cycle and partly by cytokinetic failure, and (b) a surge in the number of multinucleated cells and micronuclei-containing cells. Interestingly, pharmacological inhibition of PI3K significantly suppressed the CA phenotype[82]. Moreover, siRNA-mediated knockdown of Akt and overexpression of phosphatase and tensin homolog (PTEN) or dominant-negative Akt, abrogated the CA, implicating the involvement of PI3K signaling. Constitutively active Met caused an increase in aneuploidy in $p53^{-/-}$ cells, but not in $p53^{+/+}$ HCT116 cells, indicating that its ability to induce CIN was related to

p53 status. Thus, aberrant hepatocyte growth factor-Met signaling induces CA and CIN *via* the PI3K-Akt pathway, providing strong evidence of cross talk between oncogenic growth factor signals and CA and CIN in colon cancer.

Aurora-A signaling

Aurora-A regulates the function of centrosomes, spindles, and kinetochores for proper mitotic progression. Aurora-A overexpression frequently occurs in various cancers including colon cancer, and a link between Aurora-A overexpression and CIN has been proposed. Previous studies have found that Aurora-A overexpression in mouse mammary epithelium induces malignant transformation and CIN preceded by CA, tetraploidiza tion and premature sister chromatid segregation[83,84]. More recently, a study also found that Aurora-A is over-expressed in primary colorectal tumor cells, in the colorectal cancer stem cells (CR-CSC) fraction, and in stem cell-derived differentiated cells, compared with normal colon tissue. Interestingly, Aurora-A overexpression was functionally linked to CA in CR-CSC in this study[85]. Another study detected substantial Aurora-A overexpression in CRCs[86]. In this study, other sample features such as LOH in 2p, 5q, 17q, and 18q, the extent of CIMP and MSI phenotypes were also evaluated, and a highly significant association between Aurora-A overexpression and CIN (defined as the presence of LOH in any of the chromosomal segments) was found. In sum, deregulation of Aurora-A mediated signaling could potentially underlie CA and CIN in colon cancer.

The p120-catenin pathway

p120-catenin plays a key role in regulating the functionality of *E*-cadherin, a protein essential for the establishment and maintenance of cell-cell contacts in epithelia. Alterations in p120-catenin levels are a common event in colorectal tumors, and the distribution of p120-catenin and *E*-cadherin are coordinately regulated[87]. Another study showed that overexpression of the p120-catenin isoform 3A in HT-29 human colon adenocarcinoma cells resulted in cytoplasmic accumulation of the protein (as observed in many tumors), a reduction in cell proliferation, and a prolonged S phase associated with cyclin E stabilization. Cyclin E co-localized with cyclin-dependent kinase 2 and with p120-catenin in centrosomes during mitosis, concomitant with Thr^{199}-phosphorylation of nucleophosmin/B23. This post-translational modification of nucleophosmin has been shown to trigger the initiation of centrosome duplication. Therefore, p120-catenin-mediated accumulation of cyclin E in centrosomes may potentially induce CA in colon cancer[88].

The hSgo1 pathway

hSgo1 is one of the two human shugoshin or Sgo proteins (hSgo1 and hSgo2), that regulate sister chromatid cohesion by protecting the integrity of the multi-protein cohesin complex, and thus ensure faithful chromosome segregation during mitosis and meiosis. A study of 46 CRC cases found that hSgo1 mRNA expression was decreased in the tumor tissue in comparison with the corresponding normal tissue[89]. HCT116 cells in which hSgo1 was knocked down showed aneuploidy, micronuclei formation, increased CA and rampant mitotic catastrophe, all suggesting that hSgo1 downregulation leads to CIN in CRC cells and that hSgo1-downregulated colorectal cancers exhibit several clinicopathological characteristics of CIN, including CA.

Protein degradation *via* the Fbxw7 (or hCdc4) pathway

The ubiquitin-proteasome system plays a major role in the fine-tuned regulation of cell division by modulating the turnover of key proteins. The E3 ubiquitin ligase F-box and WD repeat domain-containing 7 (Fbxw7) or hCdc4, a member of the F-box family of proteins, which are substrate recognition components of the ubiquitin ligase SCF (Skp1-Cdc53/Cullin-Fbox-protein), mediates the ubiquitin-dependent proteolysis of several oncoproteins including cyclin E1, c-Myc, c-Jun, and Notch. Fbxw7 is the fourth most frequently mutated gene in human colorectal carcinomas. Based on the oncogenic potential of several of Fbxw7's substrates, the frequent allelic loss of the locus encoding this protein, and the demonstration that mutation of FBXW7 cooperates with p53 in mouse tumorigenesis[90], Fbxw7 was suspected to play a tumor suppressive role in human cancer. In a study that involved an extensive genetic screen of primary tumors, FBXW7 was found to be mutationally inactivated in 35% of cholangiocarcinomas, 31% of T-cell acute lymphocytic leukemias, 9% of tumors of the endometrium, 9% of colon cancers and 6% of tumors of the stomach. Approximately 43% of all mutations occurred at two Arg residues (Arg465 and Arg479) that are critical for substrate recognition[91]. Thus, Fbxw7 regulates tumorigenesis by controlling the abundance of different substrates in a dose-dependent fashion[92]. Inactivation of Fbxw7 results in an accumulation of cyclin E because Fbxw7 normally mediates the ubiquitin-dependent degradation of cyclin E. In HCT116 colon cancer cells lacking Fbxw7, the

investigators found evidence of genetic instability and a threefold increase in CA, suggesting that a decrease in the amount of active cellular Fbxw7 could potentially promote CA in colon cancer[93].

The telomerase transcriptional elements-interacting factor (TEIF) pathway

CA and telomere shortening are phenotypes that commonly occur in human cancers and are often related to dysfunction of the DNA damage repair machinery. Importantly, they are both strongly associated with genomic instability. Studies have revealed that some factors that operate in the maintenance of telomeres also participate in centrosomal homeostasis, suggesting that they are functionally linked. Telomerase transcriptional elements-interacting factor (TEIF), is one such protein that is localized to centrosomes in both physiological as well as pathological conditions and is a known transactivator of human telomerase reverse transcriptase subunit. TEIF is overexpressed in colorectal adenoma and CRC compared to normal tissue; its over-expression correlates positively with CA and tumor grade[94]. Overexpression and depletion of TEIF significantly affected centrosome status and increased mitotic abnormalities in a variety of cancer cell lines. Localization of TEIF to the centrosome was also increased by treatment with genotoxic agents and experimental telomere dysfunction, which concomitantly induced CA[95]. Thus, TEIF may be a factor linking CA and telomere dysfunction in CRC development.

Centrosomes as beacons of risk: the potential use of centrosome amplification as a prognostic biomarker and companion diagnostic

CA is well recognized as a hallmark of cancer and semi-quantitative analyses of CA have correlated this trait with tumor aggressiveness in a wide swath of cancer types[44]. Recent studies that have bolstered the notion that CA drives more aggressive tumor phenotypes through both CIN-dependent and CIN-independent mechanisms[36,96], have strongly positioned supernumerary centrosomes as potential therapeutic targets in tumors. In fact, centrosome declustering is acknowledged as a potential chemotherapeutic strategy whose edge could come from its selectivity -as only cancer cells tend to have supernumerary centrosomes and healthy cells would remain unscathed by declustering agents[97]. CA is a readout for the deregulation of multiple pathways that all culminate in the production of excessive copies or aberrant versions of this essential organelle. CA is not only recognized as a potent driver of CIN but also as a major force fueling the generation of intratumoral genetic heterogeneity[98]. Given (i) the high prevalence of CIN (over 80% of CRCs are CIN-positive) in CRC, (ii) the frequent observation of amplified centrosomes in CRCs (***Fig. 1***), and (iii) the evidence supporting that many of the signaling pathways whose deregulation has been previously implicated in induction of CA are deregulated in CRCs, it is reasonable to postulate that CA is likely to be widespread in CRCs and quantitation of CA could offer valuable information about the "aggressiveness potential" inherent in a tumor. Studies show that high-CIN colon cancer is associated with significantly poorer outcomes compared with low-CIN or high-MIN colon cancers. Quantitation of CA in CRCs can be accomplished readily using simple immunohistochemical methods that are clinically adaptable and cost-effective. In order to better distinguish between structural and numerical CA and their individual contributions towards disease aggressiveness and phenotypes and to distinguish between the impacts of the prevalence (frequency of CA or the percentage of cells exhibiting CA), and severity of amplification, more fine-grained methods of quantitating CA may need to be developed. Studies that correlate the extent of CA to tumor grade, stage and a variety of other biomarkers can be very useful to identify patients whose centrosome profiles may make them suitable candidates for centrosome-targeting therapies. In addition to serving as a prognostic biomarker, evaluation of CA could thus be used as a companion diagnostic to identify patients who might benefit from declustering drugs such as griseofulvin. Such studies could also validate the concept that CA could stand as a clinically-facile surrogate for intratumoral heterogeneity. Analysis of centrosomal profiles of CRCs could potentially uncover new subtypes of the disease that may be characterized by certain forms of centrosomal aberrations and defects. In this genomic era, one could very well be missing the low-hanging fruit-the prognostic information that could be readily garnered by characterizing the centrosomal status of tumors. The future of personalized medicine for treatment of CRC could thus hinge on unsettling, change-driving novel biomarkers like CA that go beyond the limiting confines of gene sequence-informed clinical decision-making, and allow clinicians to exploit vulnerabilities that may be conferred by the patient's unique organellar complement.

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